thickness-dependent nematic and smectic-like assembly, and nuclear component Lamin-A (LMNA), which is widely implicated in cell differentiation, was also found to be different for thin films relative to thick gels of the same E. Transcriptional profiles of many nuclear genes, including LMNA and histones, also showed a dependence on h. The changes suggest mechanical links to regulation of gene expression by matrix physics.

2389-Pos Board B375
Stem Cells Behaviour on Nano-Films of Collagen Fibrils
Irena Lambrova Ivanovska, Dennis E. Discher.
Commitment of stem cells to different lineages is regulated by many cues in their local microenvironment, including mechanical aspects of the extracellular matrix [Engler et al, Cell 2006]. We study the behavior of human mensechymal stem cells (hMSC) cultured on molecularly thin highly ordered collagen films that are transglutaminase-crosslinked or not. Cells pull on the collagen films, and their ability to deform the collagen fibrils is greatly influenced by the films' rigidity. Nanotopography and mechanics of the films are evaluated by Atomic Force Microscopy techniques (AFM) with the AFM stylus used to deform the fibrils, mimicking cellular processes of collagen remodeling. Crosslinked films require higher forces for similar plastic deformations of native collagen films. Cells cultured on different films initially respond by altering their morphology, cytoskeleton organization and nuclear shape. Mechanically anisotropic native collagen films promote strong polarization and orientation along the highly aligned fibrils whereas cells on crosslinked films flatten, spread and resembles cells undergoing osteogenesis. At least two major osteogenic markers are upregulated on such films, in contrast to cells cultured on the native collagen films. How cell and nuclear elasticity are altered in differentiating cells was also addressed in relation to expression of nuclear lamina proteins. The results suggest that the mechanics of collagen matrix - not just composition - is a major cue to nuclear state and stem cell differentiation.

2390-Pos Board B376
Leukocyte Transmigration is Mediated by Endothelial Cell Contractile Forces and Substrate Stiffness
Kimberly M. Stroka, Helim Aranda-Espinoza.
Leukocyte transmigration through the vascular endothelium is a crucial step in the normal immune response. However, in the cardiovascular disease (CVD) of atherosclerosis, an excess of leukocytes adhere to and transmigrate through the endothelium, and progression of this disease is associated with arterial stiffening and variance in mechanical force transduction. In this study we investigated the mechanics of leukocyte transmigration in an *in vitro* model of the vascular endothelium. We modeled healthy versus diseased blood vessels through manipulation of substrate stiffness using polyacrylamide gels, coated with extracellular matrix protein and plated with human umbilical vein endothelial cell (HUVEC) monolayers. The HUVEC monolayers were activated with tumor necrosis factor-alpha to mimic inflammatory conditions. We observed that leukocyte transmigration through HUVEC monolayers increases with stiffness below the endothelium, and we hypothesized that substrate stiffness changes the biophysical properties of the endothelium to produce this effect. Using an array of biophysical techniques, we first evaluated the adhesion protein expression, stiffness, morphology, cytoskeletal arrangement, and cell-substrate adhesion of HUVEC monolayers as a function of substrate stiffness; however, none of these properties could account for the transmigration behavior. We also explored the role of endothelial cell-cell adhesion and myosin light chain kinase (MLCK)-dependent endothelial cell contraction. We observed that (1) decreasing cell-cell adhesion increases transmigration on soft substrates and (2) inhibition of MLCK and endothelial cell contraction normalizes the effects of substrate stiffness by reducing leukocyte transmigration on stiff substrates without affecting transmigration on soft substrates. These results provide strong evidence that neutrophil transmigration is regulated by MLCK-mediated generation of gaps at cell borders through endothelial cell contractile forces which depend on substrate stiffness.

2391-Pos Board B377
The Origin and Limits of Natural Variation in Cell Mechanical Behavior
John M. Maloney, Krystyn Van Vliet.
Investigations of natural variation in cell mechanics within a population are essential for understanding the stochastic nature of cell deformation under applied load. Historically, a wide range of methods to measure cell stiffness have revealed a weak power law that governs the relationship between complex elastic modulus and excitation frequency; similarly, measurements of creep compliance find a corresponding power-law dependence on load application time. In both experimental regimes, researchers have reported a Gaussian distribution of power-law exponent along with a log-normal distribution of cell stiffness/compliance. However, the underlying analytical relationship between these two distributions has not yet been fully explored. Do these mechanical distributions stem from inherent variations within a cell population, from stochastic mechanisms of single-cell deformation, or both? Here, we develop this relationship to generate new predictions regarding the evolution of mechanical parameters during testing in the frequency and time domains. These predictions are in agreement with literature reports that were originally presented as empirical findings lacking theoretical explanation, as well as with our own studies of creep compliance in stem cell populations. Our work thus serves to link two fundamental findings in the cell rheology literature, produce nontrivial predictions supported by experiment, and motivate further study into constitutive laws describing the mechanical variation of living matter.

2392-Pos Board B378
Stiffness and Load-Dependent Spreading of Cell-Surface Adhesions are Emergent Properties of a Molecular Model
Sam Walcott, Sean X. Sun.
Cells plated on a 2-D substrate form adhesions with that surface. These adhesions, consisting of aggregates of various proteins, including integrins, paxillin, vinculin, talin and others, are thought to be important in mechanosensation, the process by which the cell senses and adapts to mechanical properties of the surface, such as stiffness. At a molecular level, integrins in the adhesion bind to the extracellular matrix, while the various other proteins are involved in, among other things, binding to actin filaments that, in turn, apply a load to the adhesion. Several of these proteins (e.g. talin) undergo load-dependent state transitions that are thought to be important in both the load-dependent and the surface stiffness-dependent stability of the adhesions. Based on these molecular-level observations, we consider a grossly simplified version of an adhesion, made up of "molecules" that can bind to the surface in a strain-dependent manner and can undergo a load-dependent state transition. Remarkably, we find that, in Monte-Carlo simulations of these molecules, molecular aggregates are formed in a load- and stiffness-dependent fashion that closely mimics that seen in experiment. Furthermore, we find that these adhesions exhibit three phases of growth: 1) nucleation, where small, transient molecular aggregates form; 2) maturation, where adhesions grow quadratically in time; and 3) decay, where a short steady-state is followed by adhesion disassembly. These three phases of adhesion growth are also experimentally observed. These various properties of the Monte-Carlo simulation may be simply understood by analytic calculations. We therefore conclude that many experimental observations of stiffness- and load-dependent adhesion growth are emergent properties of molecular-mechanical systems with strain-dependent surface binding and a load-dependent state transition.

2393-Pos Board B379
Membrane Feedback Controls Signaling Regulation of Cell Spreading
Padmini Rangamani, Marc-Antoine Fardin, Yuguang Xiong, Azi Lipshtat, Olivier Rossier, Michael Sheetz, Ravi Iyengar.
Cell motility and spreading are regulated by signaling from the integrin receptors. Interaction between the integrin receptors and substrates such as fibronectin triggers the activation of downstream signaling pathways, resulting in the activation of actin regulating proteins such as Arp2/3, gelsolin and profilin. We have developed an integrated model of cell spreading regulated by integrin signaling network to understand the role of signaling during fast isotropic spreading, when fibroblasts spread on fibronectin coated slides. The three dimensional stochastic spreading model was developed using differential geometry techniques and is coupled with a deterministic model for integrin signaling regulation. We find that cell spreading is a robust process and depends on signaling only for the initiation of spreading but not for maintaining the spreading dynamics. Our model further predicts that signaling dynamics in the absence of Cdc42 and WASP reduce the spreading rate to a small extent but do not affect the shape evolution of the spreading cell. These predictions were verified by experiments conducted with dominant negative Cdc42 cells and wiskostatin effects on cell spreading. Computational analyses predicted that the spreading shape evolution is controlled by the physical properties of the plasma membrane such as membrane surface load and membrane bending ridigity. Simulations from our model identified that changing these properties affects the spreading dynamics, in particular the shape evolution. In contrast, changing information flow through the cell signaling network has little effect. Overall isotropic fast spreading of fibroblasts on fibronectin-coated surfaces depends strongly on the biophysical properties of the plasma membrane and is robust to changes in the signaling dynamics.

2394-Pos Board B380
Differentiation of Hematopoietic Stem Cell Modulated by Actomyosin Forces
Jae-Won Shin, Kyle R. Spinler, Joe Swift, Dennis E. Discher.
Specificity of fate decisions during stem cell differentiation appears determined in part by biophysical processes that include cellular contractility and matrix elasticity, and we had previously demonstrated that human mesenchymal stem cells (MSCs) specify lineage based on these cues [Engler et al., Cell 2006]. Here, we show the importance of actomyosin force as a central node